UNIVERSITI PUTRA MALAYSIA

EFFECTS OF UNFERMENTED AND FERMENTED TEA OFSTROBILANTHES CRISPUS ON THE PROLIFERATIONOF DIFFERENT CANCER CELL LINES

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Chairperson : Associate Professor Asmah Rahmat, PhD

Faculty : Medicine and Health Sciences

Strobilanthes crispus ZII 109 (L) Bremek or *Saricocalix crispus* ZII 109 (L) Bremek (Acanthaceae) plant is originated from Indonesia. Nowadays, more variation of teas, other than *Camelia sinensis* tea, are produced and marketed. Since interest has now moved to other 'teas', several experimental tests were carried out in order to fulfill these objectives: to develop fermented and unfermented teas of *Strobilanthes crispus* leaves, to evaluate the sensory qualities, to evaluate the macro and micro nutrient composition, to determine the total phenolics content, and to determine the antiproliferative effects of the prepared samples on several human cancer cell lines, *i.e.* liver cancer (HepG-2), hormone-dependent breast cancer (MCF-7), non-hormone-dependent breast cancer (MDA-MB-231), ovarian carcinoma (CAOV3) and human

cervical carcinoma cell lines (HeLa). Generally, four different products were developed in this study; fermented *Strobilanthes crispus* tea (young leaves), fermented *Strobilanthes crispus* tea (old leaves), unfermented *Strobilanthes crispus* tea (young leaves), and unfermented *Strobilanthes crispus* tea (old leaves). Overall, the moisture content of all samples do not exceed 10%. Protein and fibre content of the samples are reported lower than the content in the fresh leaves. However these teas provide more carbohydrate and are high in ash levels. Calcium, sodium, potassium, magnesium, cuprum, ferum and iron are trace element present in all samples in various concentrations. Besides, the teas are also good sources of antioxidant vitamins (vitamin A, C and E), which correlate with the possible antioxidant activity. From all samples, fermented *Strobilanthes crispus* tea (old leaves) showed the highest level of vitamin A (β-carotene) value (2341.30 ± 38.09 μg/g sample), while unfermented *Strobilanthes crispus* tea (old leaves) is rich in vitamin C (ascorbic acid equivalent = 5177.88 ± 113.96 μg/g sample) and vitamin E (α-tocopherol equivalent = 555.91 ± 77.32 μg/g sample). While elemental analysis showed that carbon, oxygen, magnesium, aluminum, silicon, phosphorus, sulfur, chlorine, potassium, and calcium elements are present in all samples studied in various levels. The results might be different from the nutritional composition values because of the different mechanisms and methods of determination. The total phenolics content (ferullic acid equivalent) was found highest

in unfermented *Strobilanthes crispus* tea (old leaves) (40.93583 ± 0.70 mg/g of dried weight in methanol extract and 16.68333 ± 0.53 mg/g of dried weight in water extract). Results from the antiproliferative studies showed that the fermented *Strobilanthes crispus* tea (old leaves) methanol extract, fermented *Strobilanthes crispus* tea (old leaves) water extract, unfermented *Strobilanthes crispus* tea (old leaves) methanol extract and unfermented *Strobilanthes crispus* tea (old leaves) water extract inhibited 50 % of the MCF-7 cell growth with IC_{50} values = 23.0, 72.5, 63.0 and 80.5 µg/ml respectively, the unfermented *Strobilanthes crispus* tea (old leaves) methanol extract, unfermented *Strobilanthes crispus* tea (old leaves) water extract, fermented *Strobilanthes crispus* tea (young leaves) water extract, and fermented *Strobilanthes crispus* tea (old leaves) methanol extract, inhibited 50 % of the CAOV3 cell growth with IC_{50} values = 54.12, 57.22, 13.91 and 67.39 µg/ml respectively, while unfermented *Strobilanthes crispus* tea (old leaves) methanol extract and unfermented *Strobilanthes crispus* tea (old leaves) water extract inhibited 50% of the HeLa cell growth with IC_{50} values = 99.38 and 23.33 µg/ml respectively. On the other hand, sensory evaluation showed that the overall acceptability score was found highest for unfermented *Strobilanthes crispus* tea (old leaves), and this positively related with its ability to inhibit 50% of the proliferation of hormone-dependent breast cancer (MCF-7), ovarian carcinoma cell lines (CAOV3) and human cervical carcinoma cell lines (HeLa) with IC_{50} values less

than 100 μg/ml. Generally, all the tea infusions were satisfactorily acceptable. Nonetheless, some of the values obtained for the different parameters for *Strobilanthes crispus* teas appear to fall within the range reported for other tea leaves and, in some cases, they are higher. Coupled with favorable amounts of minerals, fiber, vitamins, the various phenolic compounds, and the satisfactorily acceptable taste, colour, flavour and aroma, these teas may present a potential use as an herbal tea worth promoting for commercialization.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN TEH *STROBILANTHES CRISPUS* KE ATAS PERTUMBUHAN SEL-SEL KANSER BERBEZA

Oleh

ARNIDA HANI BINTI TEH

Januari 2006

Pengerusi : Profesor Madya Asmah Rahmat, PhD

Fakulti : Perubatan dan Sains Kesihatan

Strobilanthes crispus ZII 109 (L) Bremek atau *Saricocalix crispus* ZII 109 (L) Bremek (Acanthaceae) berasal dari Indonesia. Kini, semakin banyak variasi teh selain daripada teh *Camelia sinensis* dihasil dan dipasarkan. Memandangkan tumpuan kini beralih kepada teh-teh jenis lain, beberapa siri ujian telah dijalankan di dalam kajian ini untuk memenuhi objektif-objektif ini; untuk menghasilkan teh hitam dan teh hijau dari daun *Strobilanthes crispus*, untuk menilai kualiti sensori, untuk menilai komposisi mikro dan makro nutrien, untuk menentukan jumlah kandungan fenolik, dan juga untuk menentukan kesan antiproliferasi sampel-sampel ini ke atas pertumbuhan beberapa sel kanser manusia, iaitu sel kanser hati (HepG-2), sel kanser payudara-bergantung-hormon (MCF-7), sel kanser payudara-tidak-bergantung-hormon (MDA-MB-231), sel kanser ovari (CAOV3) dan sel kanser

serviks (HeLa). Secara umumnya, empat produk yang berbeza telah dihasilkan; teh hitam *Strobilanthes crispus* (daun muda), teh hitam *Strobilanthes crispus* (daun tua), teh hijau *Strobilanthes crispus* (daun muda) dan teh hijau *Strobilanthes crispus* (daun tua). Berdasarkan ujian-ujian yang telah dijalankan, didapati kandungan lembapan di dalam kesemua sampel adalah tidak melebihi 10%. Kandungan protein dan serat pula direkodkan lebih rendah dadipada kandungan di dalam daun segar. Walaubagaimanapun, teh-teh ini mengandungi lebih banyak karbohidrat dan mempunyai kandungan abu yang lebih tinggi berbanding daun segar. Kalsium, sodium, kalium, magnesium, kuprum, ferum dan zat besi adalah elemen-elemen surih yang terdapat di dalam kesemua sampel dengan kepekatan yang berbeza. Selain itu, teh-teh ini juga merupakan sumber vitamin antioksidan (vitamin A, C dan E) yang baik. Daripada kesemua sampel, teh hitam *Strobilanthes crispus* (daun tua) menunjukkan nilai vitamin A (β-karoten) yang tertinggi (2341.30 ± 38.09 μg/g sampel), manakala teh hijau *Strobilanthes crispus* (daun tua) pula kaya dengan vitamin C (dengan kandungan asid askorbik = 5177.88 ± 113.96 μg/g sampel) dan vitamin E (α-tokoferol = 555.91 ± 77.32 μg/g sampel). Sementara analisis elemen menunjukkan bahawa elemen-elemen karbon, oksigen, magnesium, aluminium, silikon, fosforus, sulfur, klorin, kalium dan kalsium wujud di dalam sampel-sampel yang dikaji dengan nilai-nilai yang berbeza. Jumlah kandungan fenolik (asid ferrulik) adalah paling

tinggi di dalam teh hijau *Strobilanthes crispus* (daun tua) (40.93583 ± 0.70 mg/g berat kering di dalam ekstrak methanol dan 16.68333 ± 0.53 mg/g berat kering di dalam ekstrak air). Kajian antiproliferasi pula menunjukkan; ekstrak metanol teh hitam *Strobilanthes crispus* (daun tua), ekstrak air teh hitam *Strobilanthes crispus* (daun tua), ekstrak metanol teh hijau *Strobilanthes crispus* (daun tua) dan ekstrak air teh hijau *Strobilanthes crispus* (daun tua) merencat 50% pertumbuhan sel MCF-7 dengan nilai IC_{50} = 23.0, 72.5, 63.0 dan 80.5 μg/ml secara berturutan, manakala ekstrak metanol teh hijau *Strobilanthes crispus* (daun tua), ekstrak air teh hijau *Strobilanthes crispus* (daun tua), ekstrak air teh hitam *Strobilanthes crispus* (daun muda), dan ekstrak metanol teh hitam *Strobilanthes crispus* (daun tua) mampu merencat 50% pertumbuhan sel CAOV3 dengan nilai IC_{50} = 54.12, 57.22, 13.91 dan 67.39 μg/ml secara berturutan, sementara ekstrak metanol teh hijau *Strobilanthes crispus* (daun tua) dan ekstrak air teh hijau *Strobilanthes crispus* (daun tua) merencat 50% pertumbuhan sel HeLa dengan nilai IC_{50} = 99.38 dan 23.33 μg/ml secara berturutan. Penilaian sensori pula menunjukkan skor penerimaan keseluruhan terhadap teh hijau *Strobilanthes crispus* (daun tua) adalah yang paling tinggi, dan ini sejajar dengan keupayaannya dalam merencat sel-sel kanser MCF-7, CAOV3 dan HeLa dengan nilai IC_{50} kurang daripada 100 μg/ml. Secara keseluruhannya, penerimaan terhadap kesemua teh adalah memuaskan. Secara umumnya, nilai-nilai yang didapati bagi teh

Strobilanthes crispus untuk parameter-parameter yang berbeza adalah di dalam julat yang lebih kurang sama dengan teh *Camellia sinensis*, malahan lebih tinggi bagi sesetengah kes. Berdasarkan kandungan mineral, serat, vitamin, kandungan kompaun fenolik, dan juga peberimaan yang memuaskan terhadap rasa, warna dan aroma, teh-teh ini mempunyai potensi untuk dikomersialkan.

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I certify that an Examination Committee has met on 27th January 2006 to conduct the final examination of Arnida Hani Binti Teh on her Master of Science thesis entitled "Effects of Unfermented and Fermented Tea of *Strobilanthes crispus* on the Proliferation of Different Cancer Cell Lines" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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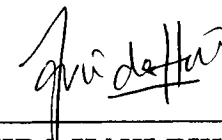
Date:
08 JUN 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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Date: 15 MAY 2006

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%	percent
˚C	degree Celcius
µg	microgram
µl	microlitre
µm	micrometer
AIDS	acquired immune deficiency syndrome
AOAC	Association of Official Analytical Chemists International
ATCC	American Type Culture Collection
BC	before Christ
CAOV3	ovarian carcinoma
DNA	deoxyribonucleic acid
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
EC	epicatechin
ECG	epigallocatechin
EGC	epicatechin gallate
EGCG	epigallocatechin gallate
g	gram
g	gravity
HCC	hepatocellular carcinoma
HCL	hydrochloric acid

HeLa	human cervical carcinoma
HEC-18	ectocervical cell
HEN-18	immortalized endocervical cell
HEN-18S	serum-adapted HEN-18
HEN-18T	transformed ectocervical cell
HepG-2	liver cancer
HPLC	high performance liquid chromatography
HPV 18	human papillomavirus type 18
i.p.	intraperitoneal
KOH	sodium hydroxide
kV	kilovolt
L	litre
LHRH	leutenizing hormone
MCF-7	hormone-dependent breast cancer
MDA-MB-231	non-hormone-dependent breast cancer
ME180	cervical cancer cell line
mg	miligram
min	minute
ml	mililitre
mm	milimeter
MOH	Ministry of Health
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid

Na_2SO_4	natrium sulfate
nm	nanometer
no	number
OD	optical density
SiHa	cervical cancer cell line
TF-1	theaflavin
TF-2a	theaflavin-3-gallate
TF-2b	theaflavin-3'-gallate
TF-3	theaflavin-3,3'-digallate
TLC	using thin layer chromatography
TMCC-1	cervical cancer cell line
UK	United Kingdom
USA	United State of America
UV	ultra violet